

Genetic Toxicology in Developing Countries: Comments and Recommendations

by John Ashby,¹ Wagida Anwar,² William W. Au,³
Ali Massoud,² and James M. Gentile⁴

Introduction

The last session of the conference on Environmental Mutagenesis in Human Populations at Risk was devoted to a panel discussion. The chairpersons of the panel were J. Ashby and A. Massoud. The moderator was J. Gentile and the panel members were R. Albertini, D. DeMarini, S. El Ghazali, R. Faris, A. T. Natarajan, Z. Riad, A. Salam, A. Shawky, M. Sorsa, R. Tennant, and M. Waters. The panel was charged with providing an overall conclusion of the conference and recommendations for future directions in the field of environmental and genetic toxicology with emphasis for developing countries. Enthusiastic exchange of ideas and suggestions were made among the panelists and between the audience and the panelists. The objectives of the discussion session were accomplished, and a summary of the discussion is presented here. The conclusions specifically address some crucial environmental and human-health problems that are encountered in developing countries such as Egypt and emphasize the need for both local and international centers of coordination. Such coordination is usually assumed to be the responsibility of government agencies, but its absence is a reality. Without such coordination, efforts to address problems with environmental mutagens are often duplicated or wasted.

Such questions came to the fore at the Cairo meeting both because many developing countries were represented and because the focus of the meeting was specifically oriented toward populations at risk, rather than to chemical mutagens per se. Specifically, it was felt that advances

in the technology associated with the detection and assessment of environmental carcinogens and mutagens should be accompanied by progress in the planning and optimal deployment of these techniques, especially in developing countries. At present, we are moving toward a situation where advanced techniques are used in a seemingly random and probably suboptimal way. The six areas considered worthy of international and national coordination are discussed below.

General Toxicology of Chemicals

Mutagenicity and carcinogenicity are highly regarded as toxic effects because they can occur in the absence of other toxic effects and long after exposure. Although these concerns are valid, they should not preclude the assessment of other more proximal toxicities. Thus, the possible teratogenicity, immunotoxicity, or embryotoxicity of a chemical should be considered with mutagenicity/carcinogenicity data when assessing overall hazard. More evident acute toxicities such as the sensitizing potential of an agent may, in fact, force human exposure levels so low that any longer term toxicities can be discounted. Such considerations can only be made on a chemical-by-chemical basis. In situations where the toxicity of a chemical is completely unknown, assessment of its mutagenicity, using the standard Salmonella mutation assay, is probably the most effective first study to conduct.

Hazard Prioritization

Chemical mutagenesis and carcinogenesis is but one possible human hazard in a given country or environment. Action on such agents should therefore proceed as part of an awareness of the major sources of pollution/disease. Once the relative priority of chemical mutagens as a hazard has been ascertained, the major sites and sources of chemical pollution should be identified. After this, consideration should be given to the practicality, costs, and benefits of taking remedial action. For example, quite

¹ICI Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire SK104TJ, England.

²Department of Community, Environmental and Occupational Medicine, Ain Shams University, Abbassia, Cairo, Egypt.

³Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX 77555.

⁴Biology Department, Hope College, Holland, MI 49423.

Address reprint requests to W. W. Au, Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX 77555.

different actions might be taken upon discovering a natural mutagen in a stable food supply as opposed to the realization that a replaceable mutagenic chemical is being used as a fungicide in food storage hoppers. In cases where a priority for action emerges and when immediate protective measures cannot be instituted, consideration should be given to the most appropriate human surveillance technique to use. Thus, having identified a mutagenic pollutant that may adversely affect human health, it would be considered more appropriate in some circumstances to mount a limited human surveillance study (to assess the likely extent of the hazard) rather than conduct full-scale animal or cell-based mutagenicity tests on the agent.

In the absence of such coordination, scarce resources may be wasted, for example, on studying the mutagenicity of mutagen A to microorganisms while human exposure to mutagen B continues unabated because of lack of adequate knowledge of relative human hazards. The problems inferred in this part of the analysis are illustrated by the fact that it is often left to individual scientists to seek out a hazardous chemical, to test it, and then to alert the relevant authorities about its properties. This is hardly the best way to prioritize hazards or to use time. It would be more effective for scientists to offer a resource for use in areas identified independently as presenting a maximum human hazard.

Another major advantage of centralizing the process of hazard recognition and hazard prioritization is that hazards can be integrated with precise measurements of human exposure, which can contribute significantly to recognizing human hazards. These measurements may then, for example, lead to priority action against a weak mutagen to which humans are chronically exposed at high dose levels, despite the presence of more potent mutagens with no human exposure in the same environment. An instance of a failure to rank hazards was provided by the extensive international debate on the possible carcinogenic hazard of nitrosation of carbaryl, although the most likely human hazard was associated with the volatile toxin methyl isocyanate used in its manufacture.

A further point is that a regulatory stance has now been taken in the western world on most commodity chemicals/pesticides/herbicides, etc. Such regulatory positions can provide a useful primary assessment of a given chemical in a new environment and should be taken into consideration by all countries. Local conditions of use may modify that initial assessment, but the alternative of ignoring the response of other countries to the same chemical should be discouraged.

Chemical Prioritization

When attempting to assess the possible impact of environmental mutagens/carcinogens on human health, it is important to know something of the range of pollutants present. Named pollutants (e.g., pesticides) are easy to recognize, but they may not be the most important agents present. Most agencies decide that endogenous mutagens

must be tolerated and that emphasis should be placed on the detection and assessment of man-made pollutants. This is acceptable so long as the implicit compromise of acceptable risk is recognized. Sometimes this compromise may be unacceptable, as in an environment known to be rich in natural nitrosamines; however, such knowledge can only come from an evaluation of chemical pollutants.

Inspecting the chemical structure of an agent for sites of actual or potential electrophilicity provides a useful primary screen for agents with an enhanced chance of being mutagenic and/or carcinogenic to several species, including humans. Such an analysis can be based on chemical knowledge or can be assisted by computerized structure-activity systems. Such structural alerts, however, only provide an indication for the need to evaluate the toxicity of an agent. They do not remove the need to conduct such tests.

The U.S. Environmental Protection Agency and the International Programme on Chemical Safety are sources of toxicity/mutagenicity data on major commodity chemicals. In addition, the European Chemical Industry Ecology and Toxicology Center has published a survey of over 2000 chemicals whose toxic status has been reviewed by one or more agencies (1). On the other hand, suppliers may be the only source of data on new chemicals. Only when sources of data have been exhausted should new studies be commissioned on a chemical. In cases where additional mutagenicity data are required before a hazard assessment can be made, it is preferable to build these studies on the internationally agreed base of assays in *Salmonella* and for clastogenicity in cultured mammalian cells. Thus, the initial assessment of a chemical in plant assays, yeast, *Drosophila* or other similar systems should be discouraged.

Rodent cancer data can be obtained from among the International Agency for Research on Cancer lists, Gold's TD₅₀ database (2), the U.S. National Toxicology Program carcinogen database, or from the suppliers of chemicals in the case of major products. Finally, updated information can also be obtained from the current scientific literature. From such an analysis, it is usually possible to assess the mutagenic/carcinogenic hazard of an agent without additional testing. Such precautionary searches will enable the limited resources available in developing countries to be used on agents of importance to the environment and for which no data exist. Unfortunately, it is common to find scarce resources in a developing country for evaluating a chemical *ab initio*, even when the agent is of known genotoxicity in developed countries and the chemical may even be banned or controlled in those countries.

The question of chemical mixtures is an ever-present problem in all countries. Not only may synergistic or antagonistic effects be produced in mixtures, but genotoxic effects considered negligible on an individual chemical basis may become significant when integrated into a mixture. Documentation of mutagenic effects in exposed populations and assessment of the mutagenicity of representative mixtures derived from an environment can provide input to hazard ranking, although international responses

to data on mixtures remain unclear.

Prioritization of chemical hazards commences with published information. Additional mutagenicity experiments and human surveillance studies flow from such analysis, but should not replace them. These considerations are of great importance in countries with limited resources and with perhaps more important human health problems that must be addressed.

Human Surveillance Studies

A wide variety of techniques for monitoring genetic damage induced in humans by environmental chemicals or environmental factors are now available. Rapid advances in improving the sensitivity of such techniques are being made, and these were reviewed at the conference. During the conference, emphasis was placed on the proper use of these techniques, and the following points emerged:

a) Populations selected for study should be influenced by a clear perception of the relative hazards in the given country or environment.

b) Specific attention should be given to the experimental design of studies, in particular, the selection of appropriate and concurrent control groups, the choice of adequate group sizes, the elimination of confounding variables, the use of coded samples for analysis, and the selection of vigorous statistical methods for the evaluation of data.

c) Attempts should be made, wherever possible, to include an intervention aspect to the study (e.g., the study of individuals before they enter the polluted environment, the follow-up of those who leave it, and monitoring the effect of selective removal of a suspect genotoxin from the environment under study).

d) The use of cytogenetic analysis in all studies on human blood is strongly recommended. This will enable a common point of comparison between studies using other end points (e.g., hemoglobin adducts, *hprt* mutations, sister chromatid exchange) and between studies performed in different countries and in time.

e) The current progress in human surveillance techniques suggests that refined methodologies will soon be available, and it will be important to reassess earlier studies using these new techniques. It is suggested that blood samples should be stored from all studies for further analysis. The possible role of the World Health Organization (WHO) and the Red Cross in this task should be explored.

f) The possible role of lifestyle variables such as deficient diet, food contaminants and infection in modulating genetic outcome from exposure to mutagens is of particular importance in developing countries.

In summary, when a major genetic hazard is recognized, an important consequence is usually that the exposed humans will be monitored to quantify that hazard. In such cases, great care should be taken to optimize the derived data by adequately designing the studies. Human surveillance studies are the last point of intervention before the recognition of human disease via epidemiological/clinical observations—as such they deserve appropriate

design and follow-up. At present, human surveillance studies tend to be conducted on limited budgets and at the discretion of individual investigators. The study of ethylene-oxide-exposed cohorts by Tates and his colleagues (3) represents an excellent model of a successful human surveillance study.

Long-Term Follow-up of Human Studies

Implicit in the use of human surveillance (genetic monitoring) techniques is that acutely induced genetic changes provide an indication of possible long-term carcinogenic or mutagenic effects. Although this provides a practical and conservative justification for such studies, *there are few data to support the underlying assumption*. A general conclusion of the conference was, therefore, that attempts should be made to initiate appropriate long-term follow-up studies—specifically, that epidemiological methods be used to correlate the outcome of surveillance studies with eventual onset of disease.

Alternatively, in some cases where a population is identified as being exposed to a possible mutagen/carcinogen, blood samples could be stored for possible future use in the case of a subsequent epidemiological study revealing an increased cancer incidence in that population. In the absence of such follow-up studies, the present human surveillance methods will have to remain simply as a means to indicate potential long-term health effects. The necessary follow-up studies will require the backing and administrative assistance of a central body such as WHO or IPCS: the conference unanimously endorsed the need for such an initiative. The further possible initiative of mounting prospective mutation epidemiology was accepted as being currently impractical; reliance will therefore have to be on data currently collected, such as abortion and cancer incidences.

An extension of the above discussion is the fact that ethical issues are raised by the conduct of human surveillance studies without adequate follow-up studies. Thus, if the implied stimulus for mounting such a human surveillance study is the need to recognize and control human exposure to a potential human carcinogen/mutagen, actions should flow from the observation of a positive effect. At present, such actions often do not follow, simply because the significance to the individual of the positive surveillance data is not clear. Carefully constructed follow-up studies would break this circle of uncertainty.

Need for International Coordination

All countries have regulatory authorities whose responsibility is to monitor the safety of key imported or domestic chemicals such as pesticides and herbicides. Often these authorities consider only potential new registrations. It is therefore easy for the situation to develop wherein extreme attention is paid to a few new chemicals while the many thousands of existing environmental chemicals are essentially ignored, unless arbitrarily selected for study

by individual investigators. A pressing need, therefore, is for a central agency in each country to be responsible for assessing the relative hazards in the environment and for prioritizing chemicals for study. Ideally, individual mutagenicity studies and human surveillance studies should be conducted considering these countrywide priorities. Further, there is an evident role for international coordination of information on the major chemicals of commerce, including agrochemicals, pesticides, and herbicides. Decisions to use a particular chemical will vary depending on local needs, but the toxicological profile of such chemicals should be internationally available as a primary input to such decisions.

Conclusions

There is a need for a standard and internationally available toxicological profile on all major man-made environmental chemicals. There is an equal need for both national and international coordination of human surveillance studies in cases where people are exposed to a known carcinogen or mutagen at levels considered likely to induce significant genetic effects. Finally, at the national level, it is necessary to consider the relative importance to

human health of discrete chemical mutagens and carcinogens. These broad recommendations are considered necessary to ensure conservation of limited resources and their focusing on problems whose solution might effectively benefit human health. At present, it is not unusual for resources to be dissipated in studying, *ab initio*, the mutagenicity of chemicals whose toxicology is well established in other countries; some such chemicals would probably also figure low on the list of priorities for action in the country in question, once such a priority list existed.

REFERENCES

1. ECETOC. Technical Report No. 30. European Chemical Industry Ecology and Toxicology Center, 1990.
2. Gold, L. S., Slone, T. H., Manley, N. B., Garfinkel, G. B., Hudes, E. S., Rohrbach, L., and Ames, B. N. The Carcinogenic Potency Database analyses of 4000 chronic animal cancer experiments published in the general literature and by the US National Cancer Institute-National Toxicology Program. *Environ. Health Perspect.* 96: 11-15 (1991).
3. Tate, A. D., Grummt, T., Törnqvist, M., Farmer, P. B., Van Dam, F. J., Van Mossel, H., Schoemaker, H. M., Osterman-Golkar, S., Uebel, C., Tang, Y. S., Zwinderman, A. H., Natarajan, A. T., and Ehrenberg, L. Biological and chemical monitoring of occupational exposure to ethylene oxide. *Mutat. Res.* 250: 283-298 (1991).